

Growth and physiology of maize plants subjected to water deficit and to different brassinosteroid and azospirillum concentrations

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Abstract

Work that demonstrates the use of brassinosteroid and azospirillum bacteria to attenuate the harmful effect of lack of water in corn is incipient. Therefore, the search for an answer that helps to understand the attenuating effect of the use of brassinosteroid and azospirillum bacteria on the physiological characteristics and growth of corn is relevant, since the water demand of the crop is high and the search for alternatives is necessary viable ways to reduce the harmful effects of water deficit. The objective of this study was to evaluate the influence of brassinosteroid and *Azospirillum brasilense* on the growth and physiology of corn plants submitted to water stress conditions. The experiment was carried out in a vegetable house with a hybrid K9606 VIP3 corn. The experiment was carried out in a vegetable house, using corn plants, the hybrid K9606 VIP3. The experimental design used was completely randomized, in a 2x2x3 factorial scheme, with six replications, totaling 72 experimental units. The first factor corresponds to two water regimes (presence and absence of water deficit). The second factor corresponds to inoculation via seed (at the time of sowing) of *Azospirillum brasilense* and absence of inoculation. And the third factor corresponds to the application of three concentrations of brassinosteroids (0, 0.3 and 0.6 μM Br). Plant height, stem diameter, number of leaves, root dry weight, leaf and stem, relative water content, chlorophyll a, b, carotenoids, sucrose, starch and total soluble carbohydrates in the leaves and roots were determined. Chlorophyll a levels were highest in water-deficient, inoculated plants with 0.3 μM brassinosteroids. In water-deficient, inoculated plants, the carbohydrate levels were higher in plants with 0.3 and 0.6 μM brassinosteroids. Among plants that were water-deficient and inoculated, plants treated with 0.6 μM brassinosteroids had the highest relative water content. Thus, it is recommended the inoculation of seeds and application of 0.6 μM of brassinosteroids together with *Azospirillum brasilense* in water-deficient maize plants.

Keywords: bacteria; climate change; hormone; *Zea mays*.

Abbreviations: AbV5 and AbV6_Straains of Azopirillum brasilense; Br_ Brassinosteroids; DAP_Days after planting; ICA_Institute of Agricultural Sciences; K9606 VIP3_Corn hybrid; KWS_Klein Wanzleben e Saat; UFRA_Federal Rural University of the Amazon; V3_Three leaves completely expanded; V7_Seven leaves completely expanded; μM _Micromol.

Introduction

Agriculture is an activity greatly influenced by climatic and meteorological conditions, where climate changes can affect the productivity of agricultural crops, threatening them or providing them with opportunities for improvement (Gornall et al., 2010). In this sense, it has been a great concern of researchers and governments around the world to study the impacts of climate change in order to understand what can be done in order to mitigate its effects and / or adapt to these changes in different human activities (Santos et al., 2011).

As the corn crop is known for its high sensitivity to water stress (Welcker et al., 2007), research on drought tolerance can provide an increase in crop growth and productivity in regions with water deficit (Li et al., 2009).

To mitigate the harmful effects caused to corn plants, several hormones are involved in modulating the response of plants to stress. Among them are brassinosteroids, which are a class of plant steroid hormones that act to elongate cells, allowing plants to grow (Baghel et al., 2019). However, its effect depends on the plant's growth stage (Freitas et al., 2015), applied concentration (Freitas et al., 2014; Wei and Li, 2016) and presence or absence of environmental stress (Tanveer et al., 2019).

Several brassinosteroid compounds, most notably brassinolide, protect plants from a range of stresses, both abiotic and biotic, and are known to regulate plant growth and increase plant productivity (Jager et al., 2008; Kutschera and Wang, 2012).

In plants such as cucumber, Arabidopsis, rice, tomato, soybean and several other species, studies of brassinosteroid applications show that this class of hormone regulates several genes in physiological and developmental processes (Anwar et al., 2018; Kanwar et al., 2017).

Work with sorghum seeds treated with brassinosteroids showed increased seedling emergence and growth under osmotic stress (Vardhini et al., 2011).

Vardhini et al., (2011) observed an increase in the emergence and growth of seedlings under osmotic stress, in studies carried out with sorghum seeds treated with brassinosteroids. In papaya, an increase in leaf area and plant dry mass was verified with the exogenous application of the regulator (Gomes et al., 2013). In the corn crop, Anjum et al., (2011), based on changes in enzymatic antioxidants and increased protein synthesis, concluded that brassinolide improves drought tolerance.

In addition to the use of hormones such as brassinosteroids, the use of nitrogen fertilizer, which is important for the growth and production of corn plants, is essential for them to express their maximum agricultural potential, since nitrogen is the nutrient most demanded by plants (Duvick, 2005).

To achieve high yields in corn, it is extremely important to analyze alternative sources of nitrogen and understand how its absorption and assimilation in plants occurs (Oliveira, 2016). Diazotrophic bacteria, such as *Azospirillum brasilense*, are capable of fixing atmospheric nitrogen where it can serve as a supplementary contribution to grass cultivation (Fibach-paldi, Burdman and Okon, 2012).

One of the main mechanisms that explains the beneficial effects of seed inoculation of plants cultivated with bacteria of the genus *Azospirillum*, despite having the potential to act as N-fixing organisms, is their ability to produce and metabolize a series of plant hormones and compounds that act directly on plant growth regulation, mainly on the root system (Bashan et al., 2004).

Plant roots are one of the main structures altered by *Azospirillum* (De-Bashan et al., 2010), this is due to the synthesis of AIA by bacteria, which promotes greater development of lateral roots, adventitious and root hairs (Cassán et al., 2020), causing an increase in the capacity of plants to absorb water and nutrients (Costa et al., 2015), as a consequence there is also an increase in their biomass (Lana et al., 2012).

Teixeira et al., (2017) verified percentage gain for plant height, in a research evaluating the efficiency of *azospirillum* in corn plants, ranging from 2.2 to 25%; root length from 32 to 71%; fresh mass of aerial part from 54 to 144%; and fresh weight of the roots reaching a percentage gain of 199%.

In view of the effects that climate change has on agricultural production, it is necessary to seek more efficient management practices in order to minimize changes resulting from the water deficit. In this context, the hypothesis of the present study is that the growth parameters and physiology of maize plants under water stress change due to the concentrations of brassinosteroids and *azospirillum*. Thus, the objective of this work was to evaluate the influence of the application of brassinosteroids and *Azospirillum brasilense* on the growth and physiology of maize plants under water stress conditions.

Results and discussion

Growth of maize plants as a function of water management, inoculation with azospirillum and brassinosteroid concentrations.

The water-deficient maize plants (Table 1) inoculated with *azospirillum* and at the highest concentration of brassinosteroids (0.6 μ M) had lower leaf number values compared to plants without inoculation (12.50 and 15.00), respectively. This study shows that uninoculated maize plants subjected to water stress conditions recorded the highest number of leaves at the lowest and highest doses of brassinosteroids. This may have occurred because the time of exposure to water deficit treatments (seven days) was not enough to reduce the number of leaves, probably because the soil remained moist, since water stress is directly linked to the availability of water in the soil (Farias, 2005).

For plant height, the treatments irrigated, without inoculation and at the highest concentration of brassinosteroids (0.6 μ M) presented higher values (64.75 cm) compared to inoculated plants (43.50 cm). This demonstrates that the joint action of brassinosteroids and *azospirillum* did not promote increases in leaf number and height of corn plants, however, the application of brassinosteroid at a concentration of 0.6 μ M, without the presence of the bacteria, provided an increase in the number of leaves of 16.7% in plants with water deficiency and 32.8% in plant height in irrigated plants (Table 1). This may be because brassinosteroids act on cell elongation, xylem differentiation, root and stem elongation, apical dominance and leaf expansion processes (Bajguaz et al., 2009). Overall, brassinosteroids effectively contribute to the tolerance mechanisms used by plants to deal with various types of abiotic stress (Larré et al., 2011).

Physiological parameters of maize plants as a function of water management, inoculation with azospirillum and brassinosteroid concentrations.

The relative water content (Table 2) in irrigated, inoculated or uninoculated plants and at all concentrations of brassinosteroids were higher than in water-deficient plants. While when comparing plants with water deficiency, the inoculated treatments and at all concentrations of brassinosteroids, presented lower values of relative water content compared to plants without inoculation. In addition, the inoculated water-deficient plants had higher water content (70.88%) at the 0.6 μ M concentration, compared to the other concentrations 56.34% (0 μ M) and 62.61% (0.3 μ M). The reduction in the relative water content observed in plants subjected to water stress was probably due to the low availability of water in the soil (Table 3). Furthermore, the transpiration process of plants leads to loss of water into the atmosphere; thus, plants wither because they cannot remove water from the soil.

Determining plant water content (%) is often used in studies that focus on investigating the adaptation of plants to drought conditions (Jones, 2007). Fioreze et al., (2011) investigated the behavior of soybean cultivars subjected to intense water deficit. The results showed that the relative water content (%) observed in genotypes subjected to intense water deficit decreased compared to well-watered plants - results similar to those observed in the present study. When observing the chlorophyll a in Table 2, the plants with water deficiency, inoculated and at a

Table 1. Number of leaves, plant height, stem diameter, root, stem and leaf dry weight based on interaction among water management, inoculation with azospirillum and brassinosteroid concentrations in maize plants.

Irrigation	Inoculation	Brassinosteroids (μM)		
		0	0.3	0.6
Number of leaves				
Irrigated	Presence	15.25Aaa*	13.75Aaa	14.25Aaa
Irrigated	Absence	14.50Aaa	14.00Aaa	14.75Aaa
Water deficit	Presence	14.00Aaa	13.75Aaa	12.50Aa β
Water deficit	Absence	15.25Aaa	12.75Aba	15.00Aaba
CV (%)	9.80			
Plant height (cm)				
Irrigated	Presence	65.00Aaa	49.25Aaa	43.50Aa β
Irrigated	Absence	71.75Aaa	55.00Aaa	64.75Aaa
Water deficit	Presence	59.25Aaa	54.75Aaa	51.25Aaa
Water deficit	Absence	64.25Aaa	48.75Aaa	60.75Aaa
CV (%)	24.20			
Stem diameter (mm)				
Irrigated	Presence	21.53Aaa	16.35Aaa	19.76Aaa
Irrigated	Absence	23.77Aaa	16.73Aaa	18.23Aaa
Water deficit	Presence	20.13Aaa	17.56Aaa	14.33Aaa
Water deficit	Absence	20.50Aaa	16.47Aaa	15.65Aaa
CV (%)	29.63			
Root dry weight (g)				
Irrigated	Presence	51.25Aaa	50.00Aaa	38.75Aaa
Irrigated	Absence	51.25Aaa	43.75Aaa	62.50Aaa
Water deficit	Presence	53.75Aaa	46.25Aaa	41.25Aaa
Water deficit	Absence	52.50Aaa	40.00Aaa	53.75Aaa
CV (%)	54.97			
Stem dry weight (g)				
Irrigated	Presence	50.00Aaa	40.00Aaa	43.75Aaa
Irrigated	Absence	60.00Aaa	45.00Aaa	40.00Aaa
Water deficit	Presence	41.25Aaa	30.00Aaa	28.75Aaa
Water deficit	Absence	37.50Aaa	33.75Aaa	45.00Aaa
CV (%)	44.26			
Leaf dry weight (g)				
Irrigated	Presence	40.00Aaa	31.25Aaa	31.25Aaa
Irrigated	Absence	42.50Aaa	31.25Aaa	36.75Aaa
Water deficit	Presence	36.25Aaa	25.00Aaa	26.25Aaa
Water deficit	Absence	36.25Aaa	28.75Aaa	31.25Aaa
CV (%)	25.41			

*Means followed by the same letter (uppercase letters for water regimes, lowercase letters for brassinosteroid doses and Greek letters for inoculation with *Azospirillum brasilense*) did not differ from each other in the Tukey test, at 5% probability level.

Table 2. Relative water, chlorophyll *a*, *b* and carotenoid contents based on interaction among water management, inoculation with *Azospirillum* and brassinosteroid concentrations in maize plants.

Irrigation	Inoculation	Brassinosteroids (μM Br)		
		0	0.3	0.6
Relative water content (%)				
Irrigated	Presence	90.29Aaa*	89.38Aa β	91.66Aaa
Irrigated	Absence	91.36Aaa	92.34Aaa	91.38Aaa
Water deficit	Presence	56.34Bc β	62.61Bb β	70.88Ba β
Water deficit	Absence	76.51Baa	77.77Baa	79.32Baa
CV (%)	2.2			
Chlorophyll <i>a</i> (mg. kg⁻¹ FM)				
Irrigated	Presence	0.038Aaa	0.018Baa	0.014Aaa
Irrigated	Absence	0.017Aaa	0.021Aaa	0.022Aaa
Water deficit	Presence	0.018Aba	0.045Aaa	0.010Aba
Water deficit	Absence	0.032Aaa	0.027Aaa	0.025Aaa
CV (%)	61.28			
Chlorophyll <i>b</i> (mg. kg⁻¹ FM)				
Irrigated	Presence	0.022Aaa	0.017Aaa	0.011Aaa
Irrigated	Absence	0.037Aaa	0.015Baa	0.018Aaa
Water deficit	Presence	0.021Aaa	0.041Aaa	0.035Aaa
Water deficit	Absence	0.044Aaa	0.052Aaa	0.029Aaa
CV (%)	64.15			
Carotenoids (mg. kg⁻¹ FM)				
Irrigated	Presence	0.15Aaa	0.15Aaa	0.12Aaa
Irrigated	Absence	0.16Aaa	0.14Aaa	0.13Aaa
Water deficit	Presence	0.12Aaa	0.15Aaa	0.13Aaa
Water deficit	Absence	0.14Aaa	0.15Aaa	0.18Aaa
CV (%)	38.91			

*Means followed by the same letter (uppercase letters for water regimes, lowercase letters for brassinosteroid doses and Greek letters for inoculation with *Azospirillum brasilense*) did not differ from each other in the Tukey test, at 5% probability level.

Table 3. Total soluble carbohydrate, sucrose and starch contents based on interaction among water management, inoculation with *Azospirillum* and brassinosteroid concentrations in maize plant leaves.

Irrigation	Inoculation	Brassinosteroids ($\mu\text{M Br}$)		
		0	0,3	0,6
Total soluble carbohydrates (mmol of Glu.g⁻¹)				
Irrigated	Presence	2.16Aba	2.45Ba α	2.21Bba
Irrigated	Absence	2.08Baba	2.21Ba β	2.01Bb β
Water deficit	Presence	2.04Bb β	3.10Aa α	3.15Aa β
Water deficit	Absence	3.82Aa α	3.17Aca	3.41Aba
CV (%)	2.97			
Sucrose (mg sucrose g⁻¹ DM)				
Irrigated	Presence	21.98Bca	26.70Ba α	23.98Bba
Irrigated	Absence	20.63Bb β	22.39Ba β	21.06Bab β
Water deficit	Presence	37.15Aa β	30.43Ac β	34.43Aba
Water deficit	Absence	38.68Aa α	33.30Aba	34.42Aba
CV (%)	3.11			
Starch (mmol of GLU/g)				
Irrigated	Presence	0.257Aa α	0.328Aa α	0.226Aa α
Irrigated	Absence	0.199Aba	0.344Aa α	0.219Baba
Water deficit	Presence	0.293Aa α	0.334Aa α	0.234Aa β
Water deficit	Absence	0.250Aba	0.356Aaba	0.482Aa α
CV (%)	25.51			

*Means followed by the same letter (uppercase letters for water regimes, lowercase letters for brassinosteroid doses and Greek letters for inoculation with *Azospirillum brasilense*) did not differ from each other in the Tukey test, at 5% probability level.

Table 4. Total soluble carbohydrate, sucrose and starch contents based on interaction among water management, inoculation with *Azospirillum* and brassinosteroid concentrations in maize plant roots

Irrigation	Inoculation	Brassinosteroids ($\mu\text{M Br}$)		
		0	0.3	0.6
Total soluble carbohydrates (mmol of Glu.g⁻¹)				
Irrigated	Presence	0.615Aba	0.734Ba α	0.656Bba
Irrigated	Absence	0.630Baba	0.661Ba β	0.599Bb β
Water deficit	Presence	0.536Bb β	0.944Aa α	0.946Aa β
Water deficit	Absence	1.037Aba	0.927Aca	1.149Aa α
CV (%)	3.32			
Sucrose (mg sucrose g⁻¹ DM)				
Irrigated	Presence	7.92Bba	9.33Ba α	8.29Bba
Irrigated	Absence	7.21Bb β	7.91Ba β	7.45Bab β
Water deficit	Presence	12.97Aa β	10.65Ac β	11.95Aba
Water deficit	Absence	13.44Aa α	11.51Aca	12.04Aba
CV (%)	3.15			
Starch (mmol of GLU/g)				
Irrigated	Presence	0.072Ba β	0.089Ba β	0.148Aa α
Irrigated	Absence	0.271Aa α	0.268Aa α	0.251Aa α
Water deficit	Presence	0.460Aa α	0.512Aa α	0.086Aba
Water deficit	Absence	0.102Ba β	0.154Aa β	0.194Aa α
CV (%)	39.88			

*Means followed by the same letter (uppercase letters for water regimes, lowercase letters for brassinosteroid doses and Greek letters for inoculation with *Azospirillum brasilense*) did not differ from each other in the Tukey test, at 5% probability level.

concentration of 0.3 μM , presented an increase of 0.027 mg. kg⁻¹ FM of chlorophyll a compared to irrigated plants under the same study condition. The water-deficient inoculated plants presented higher values of chlorophyll a at a concentration of 0.3 μM compared to the lowest and the highest concentration (0 and 0.6 μM). With regard to photosynthetic pigments (chlorophyll a) (Table 2), the use of *Azospirillum brasilense* was expected to help maintain chlorophyll levels, since inoculation with this bacterium stimulates the synthesis of new chlorophyll molecules, as shown in a study carried out with wheat (Bashan et al., 2006).

For chlorophyll b, plants with water deficiency, without inoculation and at a concentration of 0.3 μM of brassinosteroid, presented an increase of 0.037 mg. kg⁻¹ FM of chlorophyll b compared to irrigated plants under the same study condition (Table 2).

According to Li et al. (2012), the beneficial action of brassinosteroids on pigment contents resulted from the reduction of chlorophyll degradation, which led to an increase in chlorophyll a and b levels, consequently, to an increase in total chlorophyll levels, as demonstrated in studies carried out with *Chorispora bungeana* under water stress conditions and application of brassinosteroids. Studies carried out with guava seedlings with water deficit showed no significant difference in the levels of chlorophyll a, b, total chlorophyll and carotenoids (Freire et al., 2009). These results corroborate those reported by Taiz and Zeiger (2004), who observed that chlorophyll levels were not affected by environmental changes (such as water deficit), except for the effect of light and temperature, which are genetically determined.

Maize plant carbohydrates as a function of water management, inoculation with *azospirillum* and brassinosteroid concentrations.

For total soluble carbohydrates and sucrose, both in leaves (Table 3) and roots (Table 4), plants with water deficit, inoculated and at concentrations of 0.3 and 0.6 μM had higher values compared to irrigated plants. This increase in total soluble carbohydrate contents may be related to the fact that brassinosteroids play a regulatory role in carbohydrate metabolism, since the application of these hormones increases the activity of enzymes linked to sugar metabolism (Schluter et al., 2002; Yu et al., 2004) and to carbohydrate concentrations in different plant species (Schluter et al., 2002; Yu et al., 2004; Lisso et al., 2006; Dalio et al., 2011). In addition, plants subjected to water deficit recorded progressively increased sucrose levels due to sucrose biosynthesis. The increase in sucrose content probably resulted from the increased activity of sucrose phosphate synthase, since this enzyme acts on the photosynthetic cell of the cytosol to protect the integrity of membranes and proteins (Hoekstra et al., 2001) under water stress conditions.

The inoculated water-deficient plants showed increments of total soluble carbohydrates in the leaves of (1.06 and 1.11 mmol of Glu.g^{-1}) (Table 3) and in the roots of (0.408 and 0.410 mmol of Glu.g^{-1}) (Table 4) at 0.3 and 0.6 μM concentrations respectively, compared to the lowest concentration (0 μM). While for sucrose in leaves and roots of maize plants, treatments with water deficit, inoculated and in the lowest concentration of brassinosteroids (0 μM), presented higher values of sucrose compared to 0.3 and 0.6 μM concentrations. Vieira et al., (2013) reported that the cultivar UFUS Riqueza, when subjected to control conditions (1,213.75 $\mu\text{g} / \text{g}$) and stress (1,387.917 $\mu\text{g} / \text{g}$), presented the highest levels of sucrose in the primary roots. According to Sala et al. (2007), the benefits associated with inoculating maize crops with diazotrophic bacteria depend on several factors, such as plant genotype, selected strains and the interrelationship between the mentioned factors and the environment.

In the leaves of maize plants (Table 3), starch showed an increase of 0.263 mmol of GLU/g in plants with water deficiency, without inoculation and in the highest concentration of brassinosteroids (0.6 μM), compared to plants irrigated in the same study condition. Among water-deficient plants, at the 0.6 μM concentration, plants without inoculation showed an increase of 0.248 mmol of GLU/g in starch content compared to inoculated plants. In addition, water-deficient plants, without inoculation, had higher starch values at the 0.6 μM concentration compared to the lowest concentration (0 μM). According to Reis et al., (2000), the lack of response to seed inoculation with diazotrophic bacteria is often associated with the inappropriate use of strains. However, there is a consensus that the plant's genotype is the main factor for the success of the association between plant and microorganism.

For starch in the roots of maize plants, inoculation with *azospirillum* provided an increase of 0.388 and 0.423 mmol of GLU/g in starch content in plants with water deficit and in concentrations of 0 and 0.3 μM of brassinosteroid, compared to the irrigated plants. Among the treatments with water deficit, inoculated plants and at concentrations 0 and 0.3 μM , showed higher values of starch in the roots compared to plants without inoculation.

Materials and Methods

Setting

The experiment was developed in a vegetable house, which belongs to the Federal Rural University of the Amazon (UFRA), Belém City, Pará State (geographic coordinates 01° 27' 21" S, 48° 30' 16" W), from December 10th, 2019 to January 28th, 2020. In this region, the average temperature is around 26.5°C, with significant air instability and average humidity around 84% (Bastos et al., 2002).

Study design

To perform the experiment, corn seeds from the company KWS (Klein Wanzleben and Saat) were used, the hybrid (K9606 VIP3) developed by from Embrapa Amazônia Oriental. The experimental design used was completely randomized in a 2x2x3 factorial scheme, with six replications, totaling 72 experimental units. The first factor corresponds to two water regimes (presence of water deficiency and absence of water deficiency). The second factor corresponds to seed inoculation (at the moment of sowing) of *Azospirillum brasilense* and absence of inoculation. The third factor corresponds to the application of three concentrations of brassinosteroids (0.0 μM , 0.3 μM and 0.6 μM).

Experimental procedures

72 buckets with a capacity of 15 kg each were used, filled with a soil collected in the 0 to 20 cm (Samples of the superficial horizon of a Yellow Dystrophic Oxisol) depth layer at the Institute of Agricultural Sciences (ICA) at UFRA. After carrying out the soil collection, the samples were sieved to remove impurities and homogenized.

The chemical analysis of the soil was carried out for fertility purposes, which was determined in the soil laboratory of the Brazilian Institute of Analysis (IBRA), according to the methods described by Iac (2001) and Embrapa (2009), obtaining the results in Table 1.

Before sowing, the corn seeds that were inoculated with *Azospirillum* were separated. Thus, 200 g of inoculant (*Azospirillum brasilense* - Strains AbV5 and AbV6, originated from Embrapa Amazônia Oriental) were mixed in 1 ml of distilled water and immediately afterwards the seeds (peat solid corn - *Zea mays*) were added and planted. Irrigation was carried out daily, adding water until reaching the field capacity of each bucket, which was observed through the drainage at the bottom of the buckets. The corn plants were obtained from the initial germination of five seeds per pot, with subsequent maintenance of only one plant through thinning. Fifteen days after planting (DAP), the first application of brassinosteroids was performed using concentrations of 0.0 μM (Control plants, without application of brassinosteroids), 0.3 μM and 0.6 μM . The 0,3 and 0,6 μM Br (Sigma-Aldrich, USA) solutions were prepared by dissolving the solute in etlianol followed by dilution with Milli-Q water [etlianol:water (v/v) = 1:10,000] (Ahammed et al., 2013); the plants were in the V3 stage (three leaves completely expanded).

For each application, 72 ml of epnobraassinolide solution was used. Initially, 100 ml of epnobraassinolide at a concentration of 0.3 μM and 100 ml at a concentration of 0.6 μM was prepared. To the 100 ml of each concentration, 5 ml of Tween-20 was added as a surfactant. In all, two applications were made, the first at 15 DAP with plants in the V3 stage and the second at 30 DAP with plants in the V7 stage (Seven

leaves completely expanded). The application was made by applying 3 ml of epibrassinolide solution per plant, with a hand sprayer on the adaxial face (top) of the leaves of the middle third of the plants, always in the late afternoon (between 17h and 18h), in order to avoid losses by evaporation and drift.

Ten days after the last application of epibrassinolide, severe water stress was applied to the plants that would receive the stress, with total water suspension (water availability 0%). The other treatments continued to receive water until the experiment was withdrawn. Seven days after the suspension of irrigation, the plants were removed.

The collection of the plants occurred at 47 DAP at 04:30 h. The determination of the relative water content was carried out *in vivo*, selecting in the greenhouse primary leaves completely expanded from each repetition to remove the discs. Soon after the plants were separated into leaf, stem and root, the roots were washed under running water. These were stored in paper bags and placed in the forced air ventilation oven at 65°C for 48 h. After drying, they were weighed to obtain the dry mass, after which the leaves and roots were ground in a Wiley mill, and properly stored in falcon tubes where the biochemical analyzes were conducted in the Laboratory of Biodiversity Studies in Higher Plants (EBPS).

Measurement of variables

The determinations for the growth parameters were based on measurements made at the end of the experimental period, obtaining the following variables: plant height, measured from the soil surface to the apex of the plant, using a millimeter ruler, expressed in cm; stalk diameter, measured at 5 cm in height in relation to the soil surface, using a 200 mm digital caliper from Digimes, expressed in mm; number of leaves, the number of total leaves present in the plants was counted. The relative water content in leaves was also determined using the method used by Slavick, (1979). The levels of chlorophyll a, b and carotenoids were determined through the method described by Lichthenthaler, (1987). The determination of sucrose concentrations was determined according to the Van Handel method, (1968). Finally, the method used to obtain the starch and total soluble carbohydrate concentrations was according to Dubois et al., (1956).

Statistical analysis

The data were submitted to analysis of variance (ANOVA) using the F test ($p < 0.05$) and the averages obtained were submitted to the Tukey test ($p < 0.05$) using the software SISVAR version 5.4 (Ferreira, 2010).

Conclusion

The use of the plant growth-promoting bacteria *Azospirillum brasilense* via seed and the exogenous application of brassinosteroids interfere in the parameters studied in the corn crop during the occurrence of water deficit, and can be an important tool to mitigate the effects of stress on the crop.

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References

- Andrade JC de, Cantarella H, Quaggio JA (2001) Análise química para avaliação da fertilidade de solos tropicais. Accessible: https://www.iac.sp.gov.br/publicacoes/arquivos/Raij_et_al_2001_Metod_Anal_IAC.pdf
- Anjum SA, Wang LC, Farooq M, Hussain M, Xue LL, Zou CM, (2011) Brassinolide Application Improves the Drought Tolerance in Maize Through Modulation of Enzymatic Antioxidants and Leaf Gas Exchange. *Journal of Agronomy and Crop Science*, 197(3), 177–185. <https://doi.org/10.1111/j.1439-037X.2010.00459.x>
- Anwar A, Liu Y, Dong R, Bai L, Yu X, Li Y (2018) The physiological and molecular mechanism of brassinosteroid in response to stress: A review. *Biological Research*, 51(1), 1–15. <https://doi.org/10.1186/s40659-018-0195-2>
- Baghel M, Nagaraja A, Srivastav M, Meena NK, Senthil Kumar M, Kumar A, Sharma RR (2019) Pleiotropic influences of brassinosteroids on fruit crops: a review. *Plant Growth Regulation*, 87(2), 375–388. <https://doi.org/10.1007/s10725-018-0471-8>
- Bajguz A (2009) Isolation and characterization of brassinosteroids from algal cultures of *Chlorella vulgaris* Beijerinck (Trebouxiophyceae). *Journal of Plant Physiology*, 166(17), 1946–1949. <https://doi.org/10.1016/j.jplph.2009.05.003>
- Bashan Y, Bustillos JJ, Leyva LA, Hernandez JP, Bacilio M (2006) Increase in auxiliary photoprotective photosynthetic pigments in wheat seedlings induced by *Azospirillum brasilense*. *Biology and Fertility of Soils*. 42(4), 279–285. <https://doi.org/10.1007/s00374-005-0025-x>
- Bashan Y, Holguin G, De-Bashan LE (2004) *Azospirillum*-plant relationships: Physiological, molecular, agricultural, and environmental advances (1997-2003). *Canadian Journal of Microbiology*, 50(8), 521–577. <https://doi.org/10.1139/w04-035>
- Bastos TX, Pacheco NP, Nechet D, Sá TD de A (2002) Aspectos climáticos de Belém nos últimos cem anos. *Série Documentos (Embrapa Amazônia Oriental)*. 128, 31.
- Cassán F, Coniglio A, López G, Molina R, Nieves S, de Carlan CLN, Donadio F, Torres D, Rosas S, Pedrosa FO, de Souza E, Zorita MD, de-Bashan L, Mora V (2020) Everything you must know about *Azospirillum* and its impact on agriculture and beyond. *Biology and Fertility of Soils*. 56(4), 461–479. <https://doi.org/10.1007/s00374-020-01463-y>
- Costa RRGF, Quirino G da SF, Naves DC de F, Santos CB, Rocha AF de S (2015) Efficiency of inoculant with *Azospirillum brasilense* on the growth and yield of second-harvest maize1. *Pesquisa Agropecuária Tropical*. 45(3):304–311. <https://doi.org/10.1590/1983-40632015v4534593>
- Dalio RJD, Pinheiro HP, Sodek L, Haddad CRB (2011) The effect of 24-epibrassinolide and clotrimazole on the adaptation of *Cajanus cajan* (L.) Millsp. to salinity. *Acta Physiologiae Plantarum*. 33(5):1887–1896. <https://doi.org/10.1007/s11738-011-0732-x>
- de-Bashan LE, Hernandez JP, Nelson KN, Bashan Y, Maier RM (2010) Growth of Quailbush in Acidic, Metalliferous Desert Mine Tailings: Effect of *Azospirillum brasilense* Sp6 on Biomass Production and Rhizosphere Community

- Structure. *Microbial Ecology*. 60(4):915–927.
<https://doi.org/10.1007/s00248-010-9713-7>
- Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*. 28(3): 350–356.
<https://doi.org/10.1021/ac60111a017>
- Duvick DN (2005) The contribution of breeding to yield advances in maize (*Zea mays* L.). *Advances in Agronomy*. 86:83–145.
[https://doi.org/10.1016/S0065-2113\(05\)86002-X](https://doi.org/10.1016/S0065-2113(05)86002-X)
- Ferreira DF (2011) Sisvar: a computer statistical analysis system. *Ciência e Agrotecnologia*, 35(6), 1039–1042.
<https://doi.org/10.1590/s1413-70542011000600001>
- Fibach-Paldi S, Burdman S, Okon Y (2012) Key physiological properties contributing to rhizosphere adaptation and plant growth promotion abilities of *Azospirillum brasilense*. *FEMS Microbiology Letters*. 326(2):99–108.
<https://doi.org/10.1111/j.1574-6968.2011.02407.x>
- Fioreze SL, Pivetta LG, Fano A, Machado FR, Guimarães VF (2011) Comportamento de genótipos de soja submetidos a déficit hídrico intenso em casa de vegetação. *Revista Ceres*. 58(3): 342–349. <https://doi.org/10.1590/s0034-737x2011000300015>
- Freitas JAA, Marinho CS, De Jesus Freitas IL, Santos PC, Da Silva MPS, De Carvalho AJC (2015) Brassinosteróide e fungo micorrízico arbuscular na produção do porta-enxerto tangerineira “Cleópatra.” *Revista Brasileira de Ciências Agrárias*. 10(1): 54–59.
<https://doi.org/10.5039/agraria.v10i1a4708>
- Freitas SDJ, Santos PC, Berilli SDS, Lopes LC, De Carvalho AJC (2014) Brotação, desenvolvimento e composição nutricional de mudas de abacaxizeiro provenientes de gemas axilares submetidas ao brassinosteróide. *Revista Brasileira de Ciências Agrárias*. 9(1): 19–24.
<https://doi.org/10.5039/agraria.v9i1a3270>
- Gomes M de M de A, Torres Netto A, Campostrini E, Bressan-Smith R, Zullo MAT, Ferraz TM, Siqueira L do N, Leal NR, Núñez-Vázquez M (2013) Brassinosteroid analogue affects the senescence in two papaya genotypes submitted to drought stress. *Theoretical and Experimental Plant Physiology*, 25(3): 186–195.
<https://doi.org/10.1590/s2197-00252013000300003>
- Gornall J, Betts R, Burke E, Clark R, Camp J, Willett K, Wiltshire A (2010) Implications of climate change for agricultural productivity in the early twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 365(1554): 2973–2989.
<https://doi.org/10.1098/rstb.2010.0158>
- Hoekstra FA, Golovina EA, Buitink J (2001) Mechanism of plant desiccation tolerance. *Trends in Plant Science*. 6(9): 431–438. [https://doi.org/10.1016/S1360-1385\(01\)02052-0](https://doi.org/10.1016/S1360-1385(01)02052-0)
- Jager CE, Symons GM, Ross JJ, Reid JB (2008) Do brassinosteroids mediate the water stress response? *Physiologia Plantarum*. 133(2): 417–425.
<https://doi.org/10.1111/j.1399-3054.2008.01057.x>
- Jones HG (2007) Monitoring plant and soil water status: Established and novel methods revisited and their relevance to studies of drought tolerance. *Journal of Experimental Botany*. 58(2): 119–130.
<https://doi.org/10.1093/jxb/erl118>
- Kanwar MK, Bajguz A, Zhou J, Bhardwaj R (2017) Analysis of Brassinosteroids in Plants. *Journal of Plant Growth Regulation*. 36(4): 1002–1030.
<https://doi.org/10.1007/s00344-017-9732-4>
- Kutschera U, Wang Z-Y (2012) Brassinosteroid action in flowering plants: a Darwinian perspective. *Journal of Experimental Botany*. 63(10): 3511–3522.
<https://doi.org/10.1093/jxb/ers065>
- Lana M do C, Dartora J, Marini D, Hann JE (2012) Inoculation with *Azospirillum*, associated with nitrogen fertilization in maize. *Revista Ceres*. 59(3): 399–405.
<https://doi.org/10.1590/S0034-737X2012000300016>
- Larré CF, de Moraes DM, Lopes NF (2011) Qualidade fisiológica de sementes de arroz tratadas com solução salina e 24-epibrassinolídeo. *Revista Brasileira de Sementes*. 33(1): 86–94. <https://doi.org/10.1590/S0101-31222011000100010>
- Li YH, Liu YJ, Xu XL, Jin M, An LZ, Zhang H (2012) Effect of 24-epibrassinolide on drought stress-induced changes in *Chorispora bungeana*. *Biologia Plantarum*. 56(1), 192–196.
<https://doi.org/10.1007/s10535-012-0041-2>
- Li Y, Sperry JS, Shao M (2009) Hydraulic conductance and vulnerability to cavitation in corn (*Zea mays* L.) hybrids of differing drought resistance. *Environmental and Experimental Botany*. 66(2): 341–346.
<https://doi.org/10.1016/j.envexpbot.2009.02.001>
- Lichtenthaler HK, Wellburn AR (1983) Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Portland Press Ltd*.
- Lisso J, Altmann T, Müssig C (2006) Metabolic changes in fruits of the tomato dx mutant. *Phytochemistry*. 67(20): 2232–2238.
<https://doi.org/10.1016/j.phytochem.2006.07.008>
- Oliveira AR De, Simões WL (2016) Cultivares de cana-de-açúcar inoculadas com bactérias. *Energia Na Agricultura*. 31, 154–161.
- Reis VM, Baldani JJ, Baldani VLD, Dobereiner J (2000) Biological dinitrogen fixation in Gramineae and palm trees. *Critical Reviews in Plant Sciences*. 19(3): 227–247.
<https://doi.org/10.1080/07352680091139213>
- Santos RS dos, Costa LC, Sediya GC, Leal BG, Oliveira RA de, Justino FB (2011) Avaliação da relação seca/produzibilidade agrícola em cenário de mudanças climáticas. *Revista Brasileira de Meteorologia*. 26(2), 313–321. <https://doi.org/10.1590/s0102-77862011000200014>
- Schlüter U, Köpke D, Altmann T, Müssig C (2002) Analysis of carbohydrate metabolism of CPD antisense plants and the brassinosteroid-deficient cbb1 mutant. *Plant, Cell and Environment*, 25(6): 783–791.
<https://doi.org/10.1046/j.1365-3040.2002.00860.x>
- Silva FC da (2009) Manual de análises químicas de solos, plantas e fertilizantes. *Embrapa Informação Tecnológica Brasília*.
- Slavík B (1974) *Methods of studying plant water relations*. Academia (Czechoslovak Academy of Sciences).
- Tanveer M, Shahzad B, Sharma A, Khan EA (2019) 24-Epibrassinolide application in plants: An implication for improving drought stress tolerance in plants. *Plant Physiology and Biochemistry*. 135(October 2018), 295–303. <https://doi.org/10.1016/j.plaphy.2018.12.013>
- Teixeira NT, Witt L de, Filho PRR da S (2017) *Microrganismos De Regeneração Nas Propriedades Químicas Do Solo , Desenvolvimento E Produção De Milho*. *Engenharia Ambiental: Pesquisa e Tecnologia*, 14, n. 2, 72–80. <http://ferramentas.unipinhal.edu.br/engenhariaambiental/viewarticle.php?id=1499&layout=abstract>
- van Handel E (1968) Direct microdetermination of sucrose. *Analytical Biochemistry*. 22(2): 280–283.
[https://doi.org/10.1016/0003-2697\(68\)90317-5](https://doi.org/10.1016/0003-2697(68)90317-5)

- Vardhini BV, Sujatha E, Rao SSR (2011) Brassinosteroids : Alleviation of Water Stress in Certain Enzymes of Sorghum Seedlings. *Journal of Phytology*. 3(10), 38–43.
- Wei Z, Li J (2016) Brassinosteroids Regulate Root Growth, Development, and Symbiosis. *Molecular Plant*. 9(1), 86–100. <https://doi.org/10.1016/j.molp.2015.12.003>
- Welcker C, Boussuge B, Bencivenni C, Ribaut JM, Tardieu F (2007) Are source and sink strengths genetically linked in maize plants subjected to water deficit? A QTL study of the responses of leaf growth and of Anthesis-Silking Interval to water deficit. *Journal of Experimental Botany*. 58(2), 339–349. <https://doi.org/10.1093/jxb/erl227>
- Yu JQ, Huang LF, Hu WH, Zhou YH, Mao WH, Ye SF, Nogués S (2004) A role for brassinosteroids in the regulation of photosynthesis in *Cucumis sativus*. *Journal of Experimental Botany*. 55(399): 1135–1143. <https://doi.org/10.1093/jxb/erh124>